CHAPTER 5

SUMMARY AND CONCLUDING REMARKS

Traditionally, ACE inhibitors are considered vasodilatory drugs, which primarily exert their action through inhibition of (circulating) angiotensin II formation. In the last two decades, it has become clear that ACE inhibitors have other activities than simple blood pressure reduction. This class of drugs also regulates several cellular and molecular biological processes by interference with the local or tissue renin-angiotensin system. Local ACE inhibition primarily blocks the formation of angiotensin II and prevents the breakdown of bradykinin, two important tissue hormones or autacoids. The action of ACE inhibitors cannot only be explained by direct effects on angiotensin II and bradykinin synthesis, but also by indirect effects through interaction with the sympathetic nervous system, arachidonic acid cascade and EDRF. Therefore, ACE inhibitors interfere with a complex network of biological processes, which are involved in both within and between cell interactions, i.e. autocrine and paracrine mechanisms, respectively. The beneficial effects of ACE inhibitors in cardiovascular disorders can be largely attributed to these local mechanisms of action, depending on the pathophysiologic conditions and the type of cell or tissue involved. This thesis deals with the local action of ACE inhibitors on the heart and blood vessels under different circumstances (normoxia, ischaemia and congestive heart failure), both in vitro and in vivo.

5.1 **In vitro animal experiments**

In vivo, it is very difficult (if not impossible) to discriminate between local and systemic mechanisms. Therefore, the local effects of ACE inhibitors were studied in the isolated rat heart in which an active pathway for the conversion of angiotensin I into angiotensin II has clearly been demonstrated (Linz et al, 1986). Using this model, the contribution of various autocrine and paracrine mechanisms to the local action of ACE inhibitors could be studied under different conditions.

5.1.1 *The normoxic rat heart*

In the isolated rat heart, under normoxic conditions, only the sulfhydryl-containing ACE inhibitors captopril and zofenoprilat increased coronary flow to a considerable extent (appendices 1–3). The coronary vasodilatory effects of the non-sulfhydryl containing ACE inhibitors enalaprilat and ramiprilat were less pronounced after addition of concentrations which were equipotent to those of captopril and zofenoprilat with respect to inhibition of angiotensin II formation in vitro (appendix 1; van

Gilst et al, 1988; Tio et al, 1989). These results indicate that other mechanisms than inhibition of local angiotensin II formation are involved. Prevention of bradykinin breakdown may also lead to vasorelaxation, either directly or indirectly by stimulation of EDRF and/or prostaglandins. However, using a bradykinin-antagonist, only the coronary vasodilatory effect of zofenoprilat could be partially blocked, indicating that enhanced bradykinin levels do not entirely account for the vasodilatory action of sulfhydryl-containing ACE inhibitors (Tio et al, 1990c). An indirect effect through stimulation of vasodilatory prostaglandins does not seem to be involved either, since coronary vasodilation by captopril and zofenoprilat could not be blocked by the cyclooxygenase inhibitors indomethacin and acetylsalicylic acid (appendix 2; Tio et al, 1989). It is unlikely that ACE inhibitors antagonize the coronary vasoconstrictive effects of the peptide leukotrienes LTC_4 and LTD_4, which are believed to play a significant role in the regulation of coronary flow in the unstimulated isolated rat heart (appendices 3 and 4). Interaction with EDRF may therefore be the most important factor. Indeed, the false precursor of EDRF, L-NMMA, decreased the vasodilatory action of captopril in a dose-dependent manner (van Gilst et al, 1991). In the isolated rabbit aorta, it has also been demonstrated that captopril, but not enalaprilat, decreased vascular tone in a dose-dependent manner. This could be attributed to scavenging of oxygen free radicals, since the sulfhydryl containing agents L-cysteine and reduced glutathione showed similar effects (Goldschmidt et al, 1991). This observation is in keeping with those of our laboratory; like captopril and zofenoprilat, both L-cysteine and reduced glutathione increased coronary flow in the isolated rat heart (appendices 1 and 4; van Gilst et al, 1991). Indeed, in the normoxic isolated rat heart, there is plenty of oxygen free radicals, leading to an increase in coronary vascular tone. Sulfhydryl-containing ACE inhibitors may therefore decrease vascular tone by scavenging of the superoxide anion. However, the clinical significance of the sulfhydryl group can be doubted, since oxygen free radicals do not play an important role in the regulation of vascular tone under normoxic conditions in vivo. Moreover, other investigators have demonstrated that captopril does not scavenge the superoxide anion, but acts as a nonspecific antioxidant (Kukreja et al, 1990).

5.1.2 *The ischaemic rat heart*

The effects of ACE inhibitors in the isolated rat heart were also tested under ischaemic conditions (van Gilst et al, 1986; de Graeff et al, 1986). Under these conditions, ACE inhibition protected the ischaemic myocardium against cellular damage, since the overflow of markers of cellular damage, such as creatine phosphokinase and purine overflow, was significantly reduced. Probably related to this reduction in cellular damage, recovery of left ventricular function improved. Moreover, ACE inhibitors also reduced the duration and incidence of malignant reperfusion arrhythmias, which was associated with a decreased overflow of catecholamines. Several underlying mechanisms have been proposed which are related to inhibition of angiotensin II levels and/or prevention of bradykinin breakdown (Hock et

al, 1985; Linz et al, 1986; Li and Chen, 1987; Rochette et al, 1987; Schölkens et al, 1988). Cyclooxygenase inhibition attenuated the cardioprotective effects of ACE inhibitors during both ischaemia and reperfusion, suggesting that this cardioprotection is mediated by an enhanced prostacyclin synthesis (van Gilst et al, 1986; Li and Chen, 1987). Although the results are conflicting, the sulfhydryl containing ACE inhibitors were more effective than the non-sulfhydryl containing ACE inhibitors. In our laboratory, we found that ramiprilat was less potent than captopril, whereas enalaprilat showed no cardioprotective effects, except for a reduction in total purine overflow (van Gilst et al, 1986). The reason for this lack of effect was not clear. Differences in pharmacokinetics could not be ruled out. In contrast to the sulfhydryl-containing ACE inhibitors captopril and zofenoprilat, the non-sulfhydryl ACE inhibitors enalaprilat and ramiprilat are poorly distributed in the heart (Cushman et al, 1989). However, other investigators were able to show cardioprotective effects after administration of the non-sulfhydryl containing ACE inhibitors enalaprilat and ramiprilat to ischaemic rat hearts (Linz et al, 1986; Li et al, 1987). Since the results from different studies were conflicting, the relation between cardiac ACE inhibition and cardioprotection after global ischaemia in the isolated rat heart have been investigated for several ACE inhibitors (Grover et al, 1991). In contrast to fosinoprilic acid, ramiprilat and enalaprilat, only the sulfhydryl containing ACE inhibitors captopril and particularly zofenoprilat were found to be effective in this study, despite nearly complete tissue ACE inhibition after administration of all ACE inhibitors. These results suggest that cardioprotection and local ACE inhibition are not related and indicate that the antioxidant effect of the sulfhydryl moiety may play a crucial role.

At present, no definite conclusions can be drawn about the mechanism underlying the anti-ischaemic activity of ACE inhibitors in the isolated rat heart. Tissue ACE inhibition appears to be crucial during ischaemia and reperfusion. However, under certain circumstances, other factors also play an important role, especially the presence of a sulfhydryl group.

5.2 **In vivo animal experiments**

5.2.1 *Myocardial infarction*

In the closed-chest pig model, the ACE inhibitors captopril and zofenopril protected against ischaemia and reperfusion-induced myocardial tissue damage (de Graeff et al, 1987; Tio et al, 1990), whereas the non-sulfhydryl containing ACE inhibitor perindoprilat did not (appendix 5). These results suggest that the sulfhydryl group may play an important role under ischaemic conditions in vivo. However, as in the isolated rat heart, the results are conflicting. Other investigators could also demonstrate antiischaemic effects in vivo after treatment with non-sulfhydryl containing ACE inhibitors. Differences in experimental model and dosing schedule may have influenced the results of the various studies (appendix 5). Therefore, more (com-

parative) studies are needed to definitely establish the significance of the sulfhydryl group in vivo.

It is yet uncertain whether the beneficial effects observed in vivo are mediated by local mechanisms. Salvage of myocardial tissue damage after ACE inhibition may be related to its local antiadrenergic activity, since there were no differences in haemodynamic parameters between treated and control groups (de Graeff et al, 1987). A potentiation of locally generated bradykinin was suggested (de Graeff et al, 1987). This hypothesis was confirmed in the dog. In this model, low concentrations of bradykinin and ramiprilat showed a reduction in infarct size without changes in systemic haemodynamics (Martorana et al, 1990). The beneficial effect of perindopril on survival after myocardial infarction during perindopril treatment can possibly be attributed to a reduction in life-threatening arrhythmias and/or acute pump failure as a result of its local action on the heart: no haemodynamic changes were observed (appendix 5). Furthermore, low concentrations of bradykinin also reduced the severity of ischaemia-induced arrhythmias in anaesthetized dogs without affecting coronary blood flow (Vegh et al, 1991).

5.2.2 *The failing heart*

ACE inhibitors have beneficial effects on failing rat hearts after myocardial infarction. It has been demonstrated that chronic ACE inhibition preserves the responsiveness of infarcted rat hearts to beta-adrenergic stimulation (appendix 9). This effect can be explained by local biochemical and structural mechanisms. In chronic heart failure, down-regulation of beta-adrenergic receptors may be induced by an increase in (locally) released catecholamines (Brodde, 1991). Because of its antiadrenergic activity, local and/or systemic ACE inhibition may preserve the beta-adrenergic receptor function in infarcted, failing rat hearts (appendix 9). Since ACE inhibitors are also known to prevent myocardial hypertrophy and fibrosis in infarcted rat hearts, the improved cardiac function upon beta-adrenergic stimulation may also be due to prevention of structural changes in the noninfarcted myocardium (appendices 6–8). The antigrowth activity of ACE inhibitors seems to play an essential role, since low doses of these drugs prevented cardiac hypertrophy without inducing haemodynamic effects (Linz et al, 1989). These results indicate that these structural changes are independent of the haemodynamic (systemic) action of ACE inhibitors. It is yet unclear which local factor is the most important. Hypertrophy can be induced by both catecholamines and angiotensin II. The role of other trophic factors, such as prostaglandins, should also be considered. Studies with bradykinin-antagonists and cyclooxygenase inhibitors are still ongoing. The sulfhydryl group does not seem to play an important role, since both sulfhydryl and non-sulfhydryl containing ACE inhibitors prevented myocardial hypertrophy (appendices 6–8).

5.3 **Human studies**

As demonstrated in animal studies, interference with local hormonal systems may largely contribute to the beneficial effects of ACE inhibitors. The clinical significance of this local action of ACE inhibitors in patients is not yet exactly known, but it may have important therapeutic implications. For example, the vasodilatory effects of ACE inhibitors are believed to be mediated by locally released vasodilating prostaglandins. ACE inhibitors should therefore not be combined with cyclooxygenase inhibitors which are known to block the vasodilatory effects of captopril in patients with heart failure (Nishimura et al, 1989). This interaction may be clinically important, since acetylsalicylic acid is increasingly used as an antithrombotic agent in patients with coronary artery disease. These patients will be frequently treated with ACE inhibitors when left ventricular dysfunction is also present. So, the question arises whether cyclooxygenase inhibition by acetylsalicylic acid antagonizes the beneficial effects of ACE inhibitors in patients with left ventricular dysfunction. In patients with heart failure (NYHA class II-IV) who were chronically treated with an ACE inhibitor, acute administration of 300 mg carbaspirin did not influence peripheral haemodynamics (appendix 10). This may be due to the fact that this drug, in contrast to indomethacin and other cyclooxygenase inhibitors, preferentially blocks the production of thromboxane A2, while prostacyclin synthesis is reduced to a lesser extent (appendix 10). However, it is also possible that the production of vasodilating prostaglandins does not play a major role in the vasodilatory action of ACE inhibitors under conditions of chronic ACE inhibition. This is confirmed by the observation that captopril failed to increase the plasma levels of prostacyclin and PGE2 under these conditions (appendix 10). Therefore, ACE inhibitors may be safely combined with acetylsalicylic acid in patients with heart failure who are on maintenance therapy with ACE inhibitors.

5.4 **Conclusion**

In the heart, ACE inhibitors may interfere with the regulation of coronary flow, preserve membrane integrity during ischaemia and reperfusion and may beneficially influence cardiac remodeling. This has been established in experimental animal models, especially the (isolated) rat heart. This action of ACE inhibitors can be explained by its interference with the local renin-angiotensin and kallikrein-kinin systems. The exact mechanism is not yet known. Not one, but several factors seem to be involved (see figure 5).

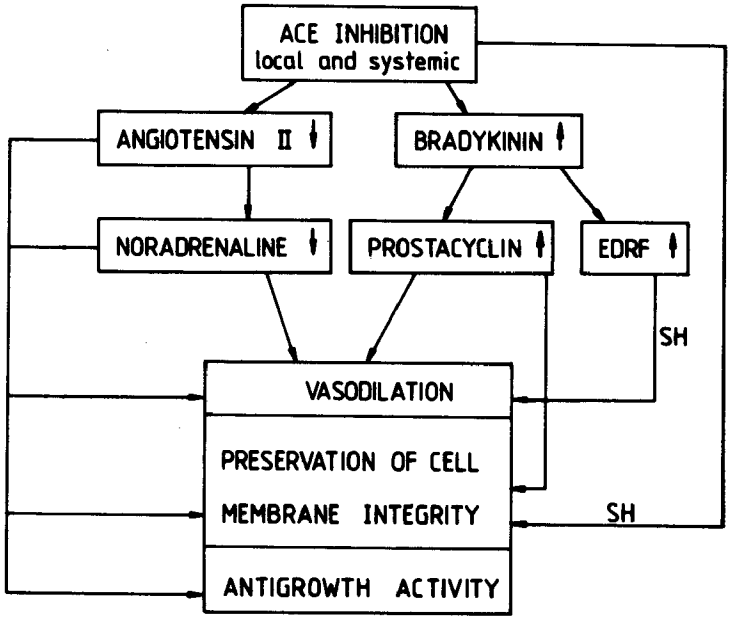

Figure 5: Tentative scheme explaining the mechanism of action of ACE inhibitors. EDRF, endothelium derived relaxing factor; SH, sulfhydryl group.

At present, no definite conclusions can be drawn about the pathophysiological significance of the cardiovascular renin-angiotensin and kallikrein-kinin systems in vivo. Animal studies suggest that these systems may play an important role in myocardial ischaemia and cardiomyopathy of overload. This may have therapeutic implications. The therapeutic value of ACE inhibitors in myocardial ischaemia is not yet clear. Neither an antianginal effect in patients with coronary artery disease, nor a reduction of myocardial tissue damage and life-threatening arrhythmias in patients with acute myocardial infarction have been demonstrated during treatment with ACE inhibitors. However, preliminary studies in patients with myocardial infarction suggest that ACE inhibitors are effective in prevention or restoration of cardiac hypertrophy and ventricular dilatation, which is possibly related to improved left ventricular function and survival. Ongoing trials will give the definite answer.